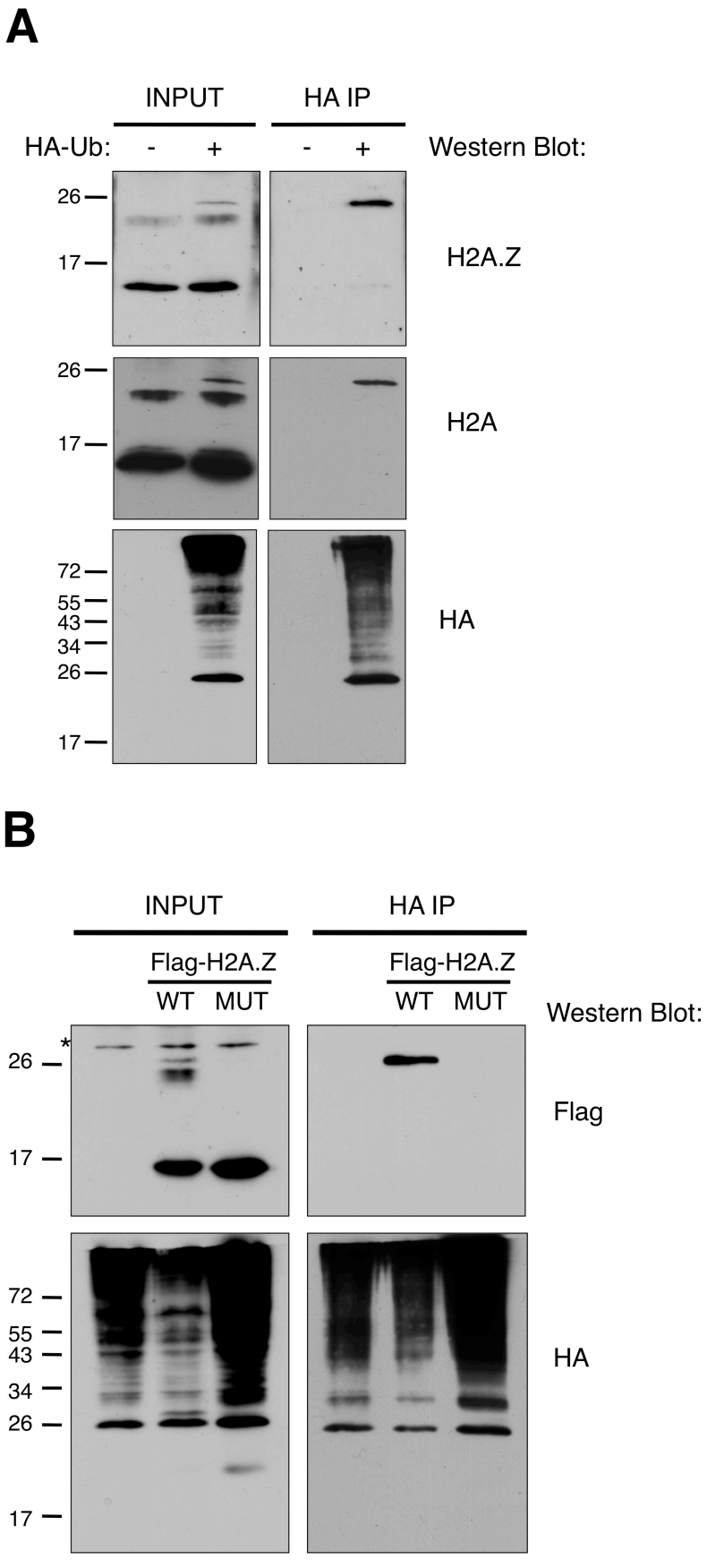
A
INPUT
HA IP
HA-Ub: - + - +
Western Blot:
26
17
H2A.Z
26
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72
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INPUT
HA IP
Flag-H2A.Z
WT MUT
Flag-H2A.Z
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Western Blot:
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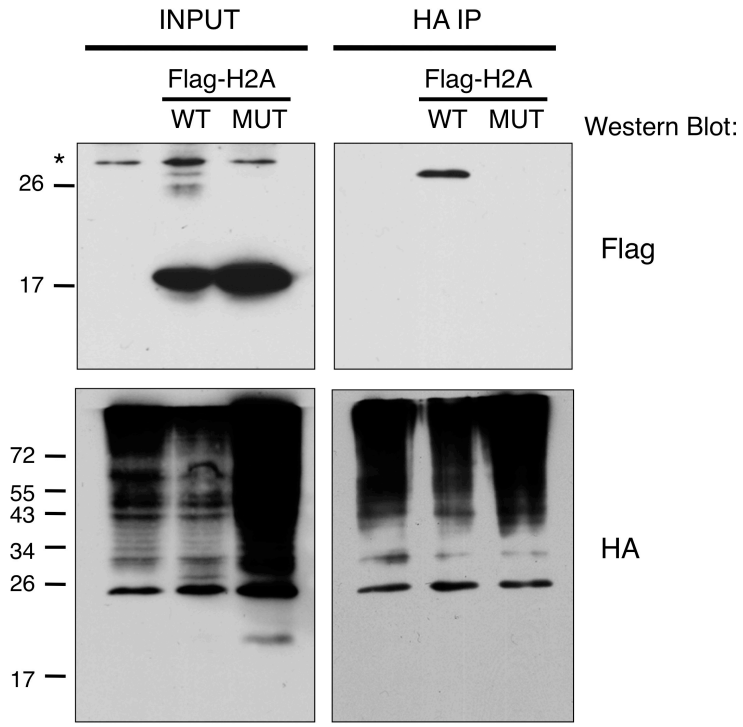
HA

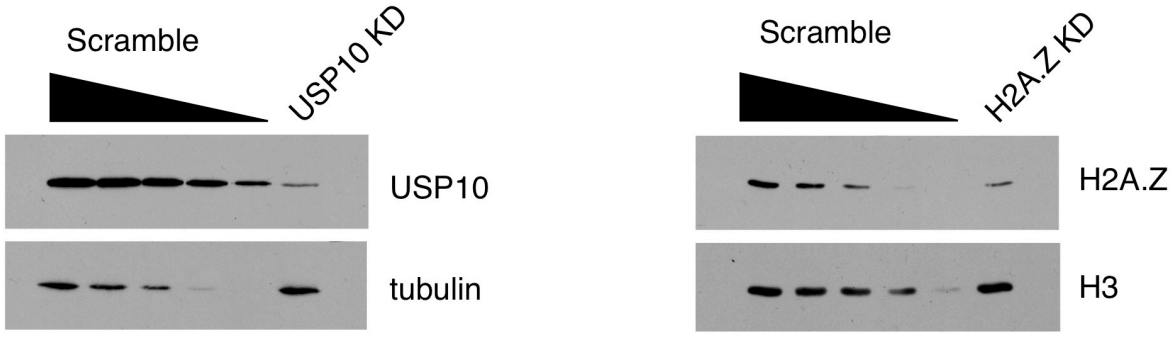
Draker_FigureS1

C



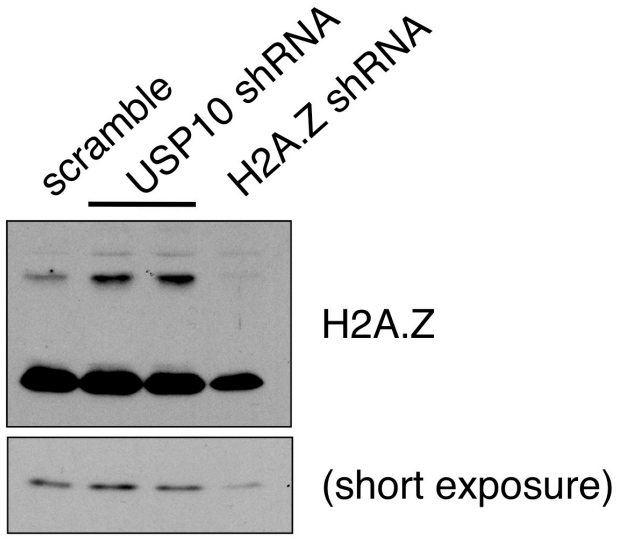
Dectection of mono-ubiquitylated H2A.Z and H2A by Western blot. (**A**) 293T cells were transiently transfected with an HA-ubiquitin expressing construct or an empty vector. Cell lysates were harvested in RIPA buffer, then used for immunoprecipitation using anti-HA monoclonal antibody conjugated to agarose beads overnight at 4°C. Following seven washes in RIPA buffer, immunoprecipitated material was eluted by boiling in 2X sample buffer, then used for Western blotting using antibodies against H2A.Z, H2A or HA. In the input fraction, both H2A.Z and H2A antibodies recognize the unmodified forms (lower band) as well as the mono-ubiquitylated forms of the histones (single upper band in lane 1). A second band, with reduced mobility, corresponding to the HA-ubiquitylated histone, is detected only in the sample from cells expressing HA-ubiquitin. Furthermore, this additional band is the only band detected in the IP'ed fraction from HA-ubiquitin expressing cells (lane 4). HA blotting displays a characteristic smear resulting from the conjugation of HA-ubiquitin to various cellular proteins **(B & C)** As in **(A)**, cells were transiently transfected with HA-ubiquitin alone, or co-transfected with Flag-tagged versions of either wild type (WT), or non-ubiquitylatable mutant versions (MUT) of H2A/H2A.Z where the C-terminal lysines have been mutated to arginines. Again, input material and HA-immunoprecipitated material was used for Western blotting, using an anti-Flag antibody or anti-HA antibody. The Flag antibody detects two bands of slower mobility only in samples expressing both the Flag-tagged histone and HA-ubiquitin. For both H2A.Z and H2A, mutation of the C-terminal lysines eliminates the detection of the slower migrating bands, further supporting the identity of the bands as ubiquitylated histones. The asterisk, "*", marks a non-specific band detected by the Flag antibody.

Draker_FigureS2



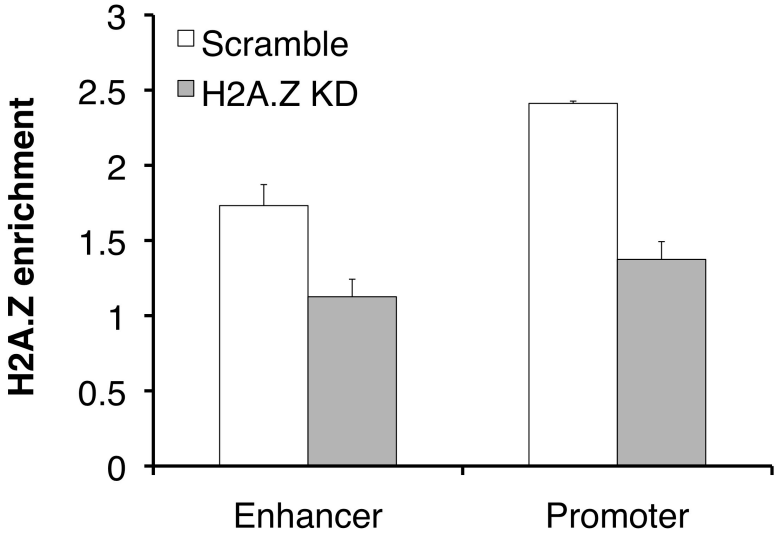
Quantification of USP10 and H2A.Z protein in LNCaP knockdown cells. Whole cell lysates from LNCaP cells with stable knockdown of USP10, H2A.Z or expressing a scrambled control shRNA were used for Western blot analysis of US10 and H2A.Z protein levels. Decreasing amounts of the Scramble control sample were loaded in order to estimate the decrease in USP10 and H2A.Z protein levels. The loading of the Scramble sample, from left to right, was as follows, in lanes 1-5: 100%, 80%, 60%, 40%, 20%. Tubulin and H3 Westerns are shown as loading controls. Note that equal loading is demonstrated in lanes 1 & 6 for both panels. USP10 protein levels in the USP10 knockdown cells are less than 20% of the control cells (compare lanes 5 & 6, left panel). H2A.Z protein levels are estimated at 50% compared to the Scramble control (compare lanes 3 & 6, right panel).

Draker_FigureS3



Western blot analysis of H2A.Z in LNCaP stable cells. Whole-cell lysates from LNCaP cells stably expressing USP10 shRNA, H2A.Z shNRA or a scrambled conrol sequence were used for Western blot analysis using an H2A.Z antibody, as described in Materials and Methods. As described in the main text (Figure 4B), knockdown of USP10 in LNCaP cells leads to an increase in H2A.Zub1 (upper band, centre two lanes) compared to the scramble control. In H2A.Z knockdown cells, a reduction in both the upper (H2A.Zub1) and lower bands (H2A.Z unmodified) are observed. The short exposure displays the lower band of the H2A.Z blot only. * Note that the USP10 shRNA sample was loaded in duplicate.

Draker_FigureS4



H2A.Z knockdown cells display a specific reduction of H2A.Z signal at the PSA gene. H2A.Z ChIP was performed as described in Materials and Methods, using unstimulated Scramble- or H2A.Z shRNA-expressing stable LNCaP cells. Compared to the Scramble-expressing cells, H2A.Z KD cells have a reduced H2A.Z signal at both the promoter and enhancer regions of the PSA gene. The ~ 50% reduction is consistent with the 50% knockdown of total H2A.Z protein levels observed in the stable LNCaP cells.